Blood levels of folate over time, current US levels, and differences between assessment methods

Christine M Pfeiffer, PhD

Chief, Nutritional Biomarkers Branch

NTP/NIEHS Expert Panel Meeting "Identifying Research Needs for Assessing Safe Use of High Intakes of Folic Acid"

May 11-12, 2015 in Bethesda, MD

National Center for Environmental Health

Division of Laboratory Sciences

Disclosure

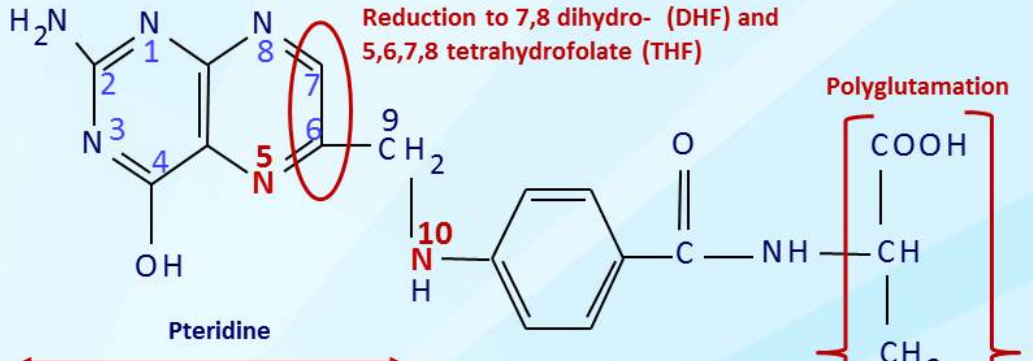
Nothing to disclose

Presentation outline

- **Assays for serum and red blood cell (RBC) folate**
  - Brief overview of available methods
  - Issues with (lack of) comparability of data
  - Issues with cutoff values
- **Serum and RBC folate status pre- vs. post-fortification**
- **Current US blood folate levels**
  - Post-fortification concentrations of serum and RBC folate
  - Post-fortification prevalence estimates of low blood concentrations
  - Post-fortification concentrations of serum folate forms, including unmetabolized folic acid (UMFA)
  - Factors associated with higher UMFA concentrations

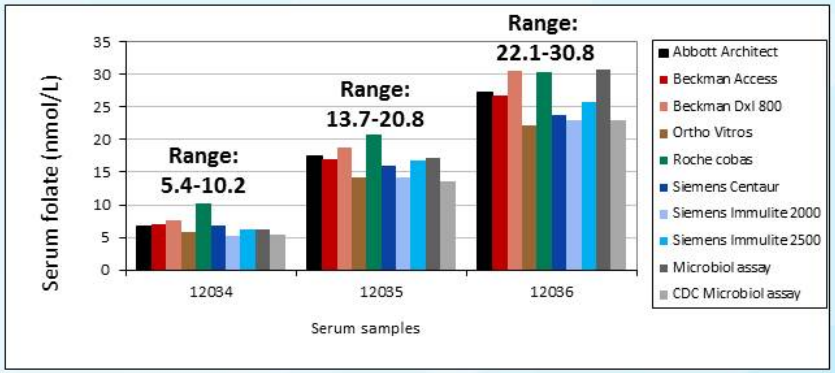
ASSAYS FOR SERUM AND RBC FOLATE

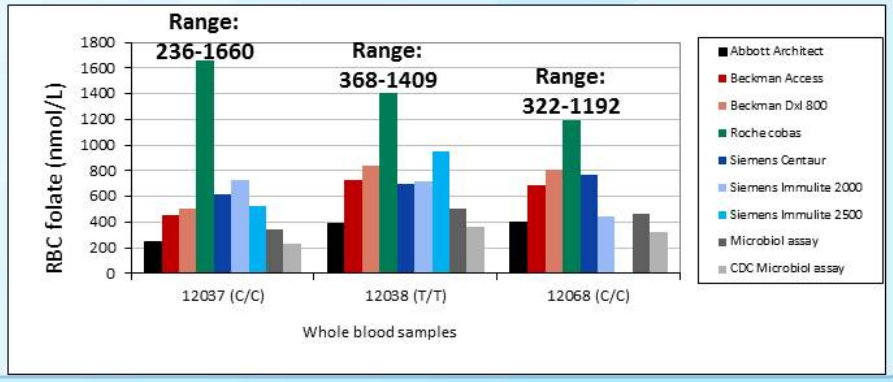
Folate structure allows variations in three areas



Coenzyme	Substituent at N-5	N-10
5-MethylTHF	CH3	
5,10-MethenylTHF	=CH	
5,10-MethyleneTHF	CH2	
5-FormylTHF	CHO	
10-FormylTHF		CHO
THF	H	H

Serum and RBC folate – 2012 UK NEQAS PT program





Main laboratory methods for serum and RBC folate

Method type	Advantages	Disadvantages
Microbiologic assay (MBA) for total folate	• Small sample volume • Inexpensive • Suited for low resource setting • Measures all biologically active forms approximately equally (however, calibration with 5-methylTHF generates lower results than calibration with folic acid)	• Relatively laborious and takes 2 days to result • Replicates needed due to higher imprecision • Multiple dilutions needed due to limited linear range • Inhibited by presence of antibiotics or antifolates
Competitive protein binding assay (CPBA) for total folate	• User friendly and minimum operator involvement • High sample throughput • Suited for clinical setting • Generally good precision (~5%)	• Questionable accuracy due to different affinities of folate forms to FBP • Less suited for long-term studies due to potential lot-to-lot variability • Matrix effects likely with sample dilution
Chromatography-based assay for folate (various detectors; recently MS/MS)	• Information on folate vitamers • Highly selective and specific • Good analytical sensitivity and precision • Suited for research setting	• Expensive instrumentation and technical service, experienced operator • Complex sample extraction/clean-up • Conversion of polyglutamates to monoglutamates needed for whole blood • Summation of folate forms to total folate

Pfeiffer et al. – Folate methods chapter in Bailey's Folate in Health and Disease, 2nd ed.

Folate methods and data in NHANES

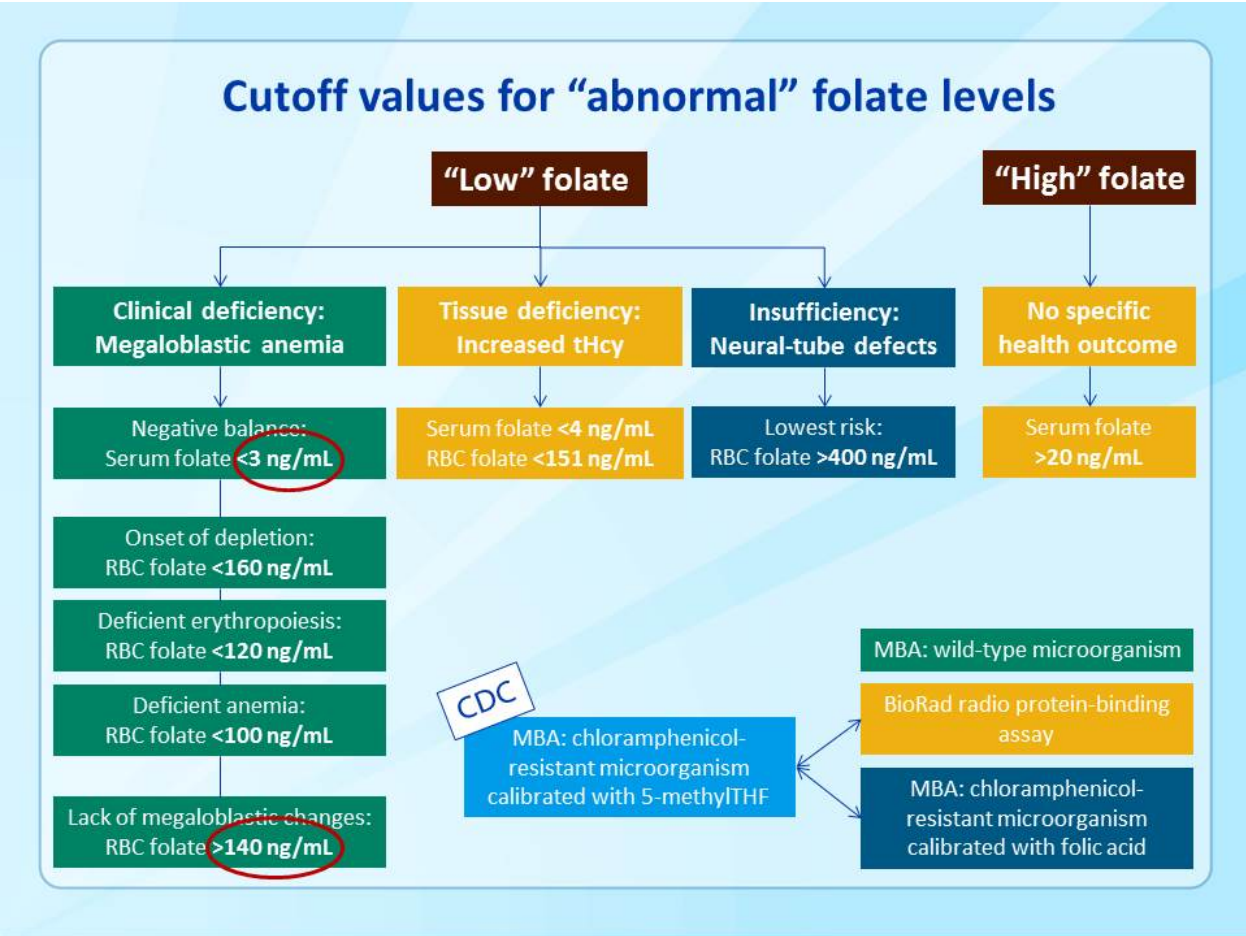
NHANES	Serum folate	RBC folate
Pre-fortification		
1988-1994	BioRad RIA	BioRad RIA
Post-fortification		
1999-2000	BioRad RIA	BioRad RIA
2001-2002	BioRad RIA	BioRad RIA
2003-2004	BioRad RIA	BioRad RIA
2005-2006	BioRad RIA	BioRad RIA
2007-2008	MBA (LC-MS/MS)	MBA
2009-2010	MBA	MBA
2011-2012	LC-MS/MS	MBA
2013-2014	LC-MS/MS	MBA
2015-2016	LC-MS/MS	MBA

Converted to MBA-equivalent data
Pfeiffer et al. J Nutr 2012

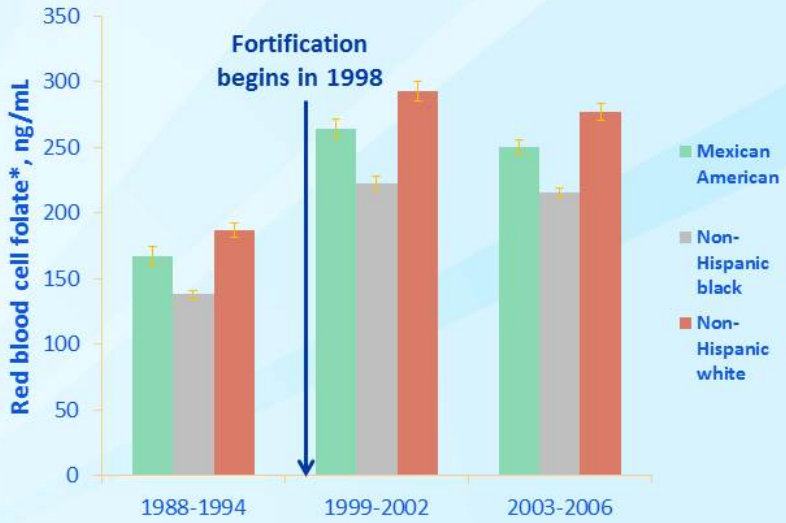
MBA approx equivalent to LC-MS/MS for serum folate
Fazili et al. Clin Chem 2007
Yetley et al. AJCN 2011

Folate methods and data in the literature

- MBA, CPBA, chromatography-based – little information on how the various methods compare at any given point in time
- MBA:
  - Limited number of labs using this assay
  - Assay results may vary depending on microorganism (antibiotic-resistant vs. wild type) and calibrator (folic acid vs. 5-methylTHF vs. 5-formylTHF)
  - Most comprehensive comparison data: *Pfeiffer et al. J Nutr 2011*
  - 2015 CDC Folate Round Robin for microbiologic assay labs
- CPBA:
  - Comparison data from a few studies, but question whether assay changed over time (*Gunter et al. Clin Chem 1996; Pfeiffer et al. Clin Chem 2001; Owen et al. Am J Clin Pathol 2003; Clifford et al. J Nutr 2005*)
- LC-MS/MS:
  - No comparison data available
  - 2015 CDC Serum Folate Round Robin for LC-MS/MS labs


Cutoff values for "abnormal" folate levels
"Low" folate
"High" folate
Clinical deficiency: Megaloblastic anemia
Tissue deficiency: Increased tHcy
Insufficiency: Neural-tube defects
No specific health outcome
Negative balance: Serum folate <3 ng/mL
Serum folate <4 ng/mL RBC folate <151 ng/mL
Lowest risk: RBC folate >400 ng/mL
Serum folate >20 ng/mL
Onset of depletion: RBC folate <160 ng/mL
Deficient erythropoiesis: RBC folate <120 ng/mL
Deficient anemia: RBC folate <100 ng/mL
Lack of megaloblastic changes: RBC folate >140 ng/mL
CDC
MBA: chloramphenicol-resistant microorganism calibrated with 5-methylTHF
MBA: wild-type microorganism
BioRad radio protein-binding assay
MBA: chloramphenicol-resistant microorganism calibrated with folic acid

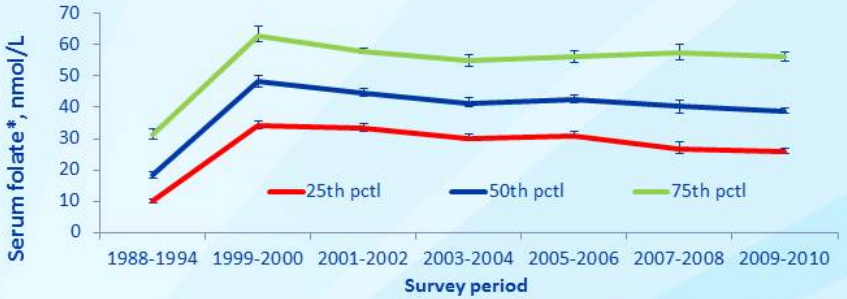
SERUM AND RBC FOLATE STATUS PRE- VS. POST-FORTIFICATION

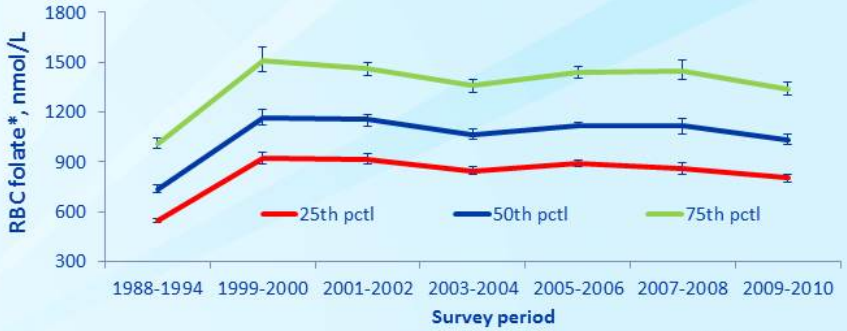


*BioRad data

CDC's 2012 Second National Nutrition Report at www.cdc.gov/nutritionreport

Post-fortification serum and RBC folate concentrations were ~2.5x and 1.5x pre-fortification concentrations, respectively

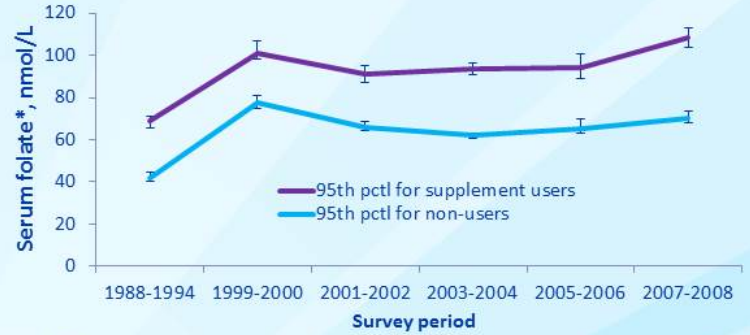


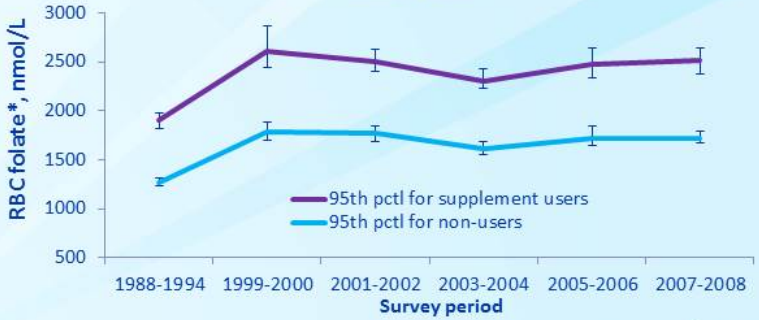


* MBA-equivalent data

Pfeiffer et al. J Nutr 2012

Higher serum and RBC folate 95th percentile concentrations for supplement users vs. non-users

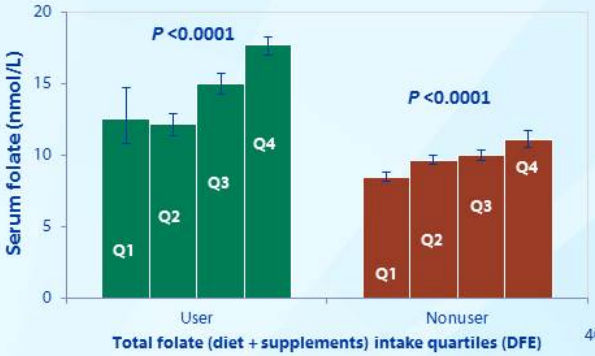


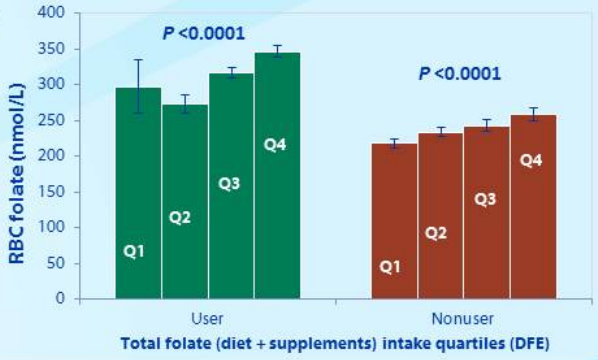


* MBA-equivalent data

Pfeiffer et al. J Nutr 2012

Relationship between blood folate concentrations and total vitamin intake quartiles in adult supplement users and non-users NHANES 2003-2006





*BioRad data

CURRENT BLOOD FOLATE LEVELS

British Journal of Nutrition, page 1 of 13 doi:10.1017/S0007114515001142

Folate status and concentrations of serum folate forms in the US population: National Health and Nutrition Examination Survey 2011–2

Christine M. Pfeiffer[1]*, Maya R. Sternberg[1], Zia Fazili[1], David A. Lacher[2], Mindy Zhang[1], Clifford L. Johnson[2], Heather C. Hamner[3], Regan L. Bailey[4], Jeanne I. Rader[5], Sedigheh Yamini[5], R. J. Berry[3] and Elizabeth A. Yetley[4]

[1]*National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA*
[2]*National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD, USA*
[3]*National Center for Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA, USA*
[4]*Office of Dietary Supplements, National Institutes of Health, Bethesda, MD, USA*
[5]*Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA*

U.S. Women of Childbearing Age Who Are at Possible Increased Risk of a Neural Tube Defect-Affected Pregnancy Due to Suboptimal Red Blood Cell Folate Concentrations, National Health and Nutrition Examination Survey 2007 to 2012

Sarah C. Tinker*[1], Heather C. Hamner[2], Yan Ping Qi[1], and Krista S. Crider[1]

Current folate status in the US population
NHANES 2007 – 2012

Survey period	Serum folate (nmol/L)	RBC folate (nmol/L)
2007 – 2008	39.5 (37.7 – 41.3)	1120 (1070 – 1160)
2009 – 2010	38.2 (37.2 – 39.3)	1040 (1010 – 1070)
2011 – 2012	41.4 (40.1 – 42.9)	1050 (1010 – 1090)

WHO cutoff:	10 nmol/L	340 nmol/L	906 nmol/L
Survey period	Serum folate <13.7 nmol/L	RBC folate <624 nmol/L	RBC folate <748 nmol/L
2007 – 2008	3.5%	7.6%	22.8% (women 12-49 y)
2009 – 2010	3.9%	9.4%	
2011 – 2012	1.1%	9.0%	

Pfeiffer et al. Br J Nutr 2015

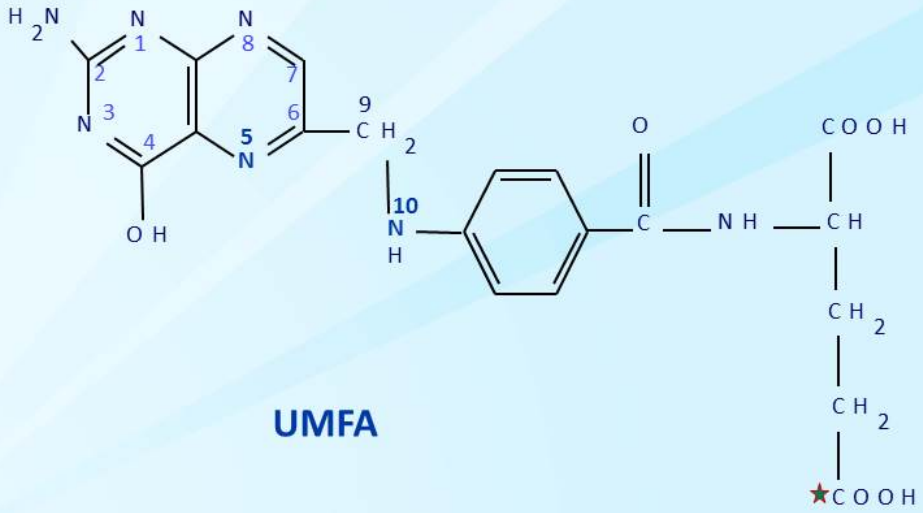
Tinker et al. 2015

Current blood folate concentrations NHANES 2011-2012

- NHANES 2011-2012 provides the first data on serum folate forms for persons 1 y and older by demographic and selected physiologic and lifestyle variables
- Concentrations of 5-methylTHF (100%), UMFA (99.9%), MeFox (98.8%), and THF (85.2%) mostly detectable
- 5-FormylTHF (3.6%) and 5,10-methenylTHF (4.4%) rarely detected
- Contribution to total folate: 5-methylTHF (86.7%), UMFA (4%), non-methylfolate (4.7%), MeFox (4.5%)
- All biomarkers showed higher concentrations with recent folic acid-containing supplement use

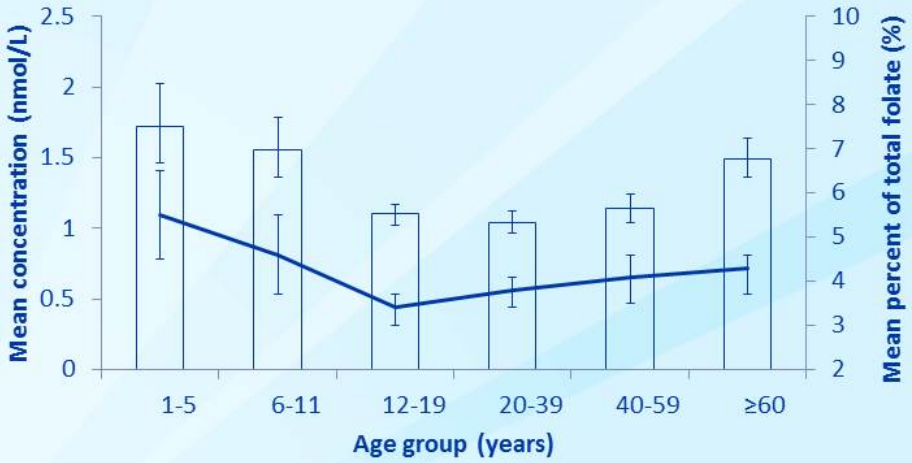
Pfeiffer et al. Br J Nutr 2015

SERUM UNMETABOLIZED FOLIC ACID CONCENTRATIONS



UMFA

Serum UMFA contributed 4% to total folate, NHANES 2011-2012



Concentrations varied with age, sex, race-ethnicity, fasting status, eGFR, BMI, BSA, serum cotinine, alcohol intake, and folic acid supplement use

Pfeiffer et al. Br J Nutr 2015

The Journal of Nutrition. First published ahead of print December 10, 2014 as doi: 10.3945/jn.114.201210.

The Journal of Nutrition
Nutritional Epidemiology

Unmetabolized Folic Acid Is Detected in Nearly All Serum Samples from US Children, Adolescents, and Adults[1–4]

Christine M Pfeiffer,[5]* Maya R Sternberg,[5] Zia Fazili,[5] Elizabeth A Yetley,[6] David A Lacher,[7] Regan L Bailey,[6] and Clifford L Johnson[7]

[5]National Center for Environmental Health, CDC, Atlanta, GA; [6]Office of Dietary Supplements, NIH, Bethesda, MD; and [7]National Center for Health Statistics, CDC, Hyattsville, MD

- Prevalence of UMFA >1 nmol/L was 33% overall and 21% in fasting (≥8 h) adults in NHANES 2007-2008
- UMFA >1 nmol/L was largely but not entirely explained by fasting status and by total folic acid intake from diet and supplements

Pfeiffer et al. J Nutr 2015

Summary

- Folate assays have not yet been standardized and results are not comparable across assays or laboratories
- Fortification has significantly increased blood folate levels in the US population
- Post-fortification prevalence of deficient blood folate levels is <10%
- Post-fortification blood folate levels have been fairly constant over a period of ~15 years
- Post-fortification detection of serum UMFA is nearly ubiquitous and concentrations >1 nmol/L are largely but not entirely explained by fasting status and by total folic acid intake from diet and supplements

Acknowledgments

Staff of the Nutritional Biomarkers Branch

Colleagues at NCHS/NHANES, NIH/ODS, and FDA

Questions?

For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333
Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info

National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention
Division of Laboratory Sciences